AWARD NUMBER: W81XWH-13-2-0089

TITLE: Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON)

PRINCIPAL INVESTIGATOR: Sherrill J. Slichter, MD

CONTRACTING ORGANIZATION: Bloodworks Northwest
Seattle, WA 98104

REPORT DATE: December 2017

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
December 2017	Final	26-SEP-13 to 25-SEP-17

4. TITLE AND SUBTITLE
Extended Storage of Pathogen-Reduced Platelet Concentrates

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-2-0089

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sherrill J. Slichter, MD

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Bloodworks Northwest (formerly Puget Sound Blood Center)
921 Terry Ave
Seattle, WA 98104

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
This grant pertains to finding novel approaches for storage of platelets for transfusion. Our project proposes to determine the efficacy of using a pathogen inactivation technique (Mirasol) coupled with a platelet additive solution (PAS) to extend the life of stored platelets. Our project also aims to determine how long acceptable platelet viability can be maintained in platelets stored at 4°C.

15. SUBJECT TERMS
bleeding, extended storage, hemorrhage, hemostasis, InterSol, Mirasol, pathogen inactivation, pathogen reduction technology, platelet additive solution, platelet recovery and survival, platelet storage, platelet storage solution, platelets, thrombocytopenia, transfusion, whole blood

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	21	19b. TELEPHONE NUMBER *(include area code)*

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Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON)
(Previously Pathogen-Reduced, Plasmalyte-Extended Stored Platelets)
Grant Number 13335012
Annual Report
26-SEP-2015 to 25-SEP-2016

INTRODUCTION: The purpose of this project is to find better ways to store platelets for patients that need platelet transfusions. A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research study, we are interested in evaluating the novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on platelet viability and function following transfusion.

KEY WORDS: 4°C storage, bleeding, cold storage, extended storage, hemorrhage, hemostasis, Isoplate, InterSol, pathogen inactivation, pathogen reduction, pathogen reduction technology, PRT, platelet additive solution, PAS, platelet recovery and survival, platelet storage, platelet storage solution, platelets, refrigerated storage, thrombocytopenia, transfusion, whole blood

OVERALL PROJECT SUMMARY: The following specific aims were described in the original statement of work, Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON).

1. Determine the optimum conditions for extended storage of autologous platelet concentrates in a platelet additive solution (PAS).
2. Evaluate the effects of Mirasol treatment of autologous whole blood (WB) on extended storage of PAS-stored platelet concentrates prepared from treated WB.
3. Determine the post-transfusion recovery and survival of pre-storage pooled extended stored platelet concentrates prepared from Mirasol-treated WB given to thrombocytopenic patients.

An evaluation of changes in the structural and functional properties of platelets stored as whole blood under refrigeration [Assessment of Whole Blood Cold Stored Platelets (Brrr Study)] was completed in 2015. Results of this trial were submitted previously and are included here. See 'Final Report - Storage of Platelets in Whole Blood at 4°C.'

From January 2016 to April 2017 we evaluated apheresis platelets stored at 4°C either in a platelet additive solution, such as InterSol or Isoplate, or stored in plasma. The protocol, entitled Cold Apheresis Platelets in Isoplate (CAPI), called for an apheresis platelet unit to be collected from a healthy subject and divided into two units. One half of the split unit was stored in plasma at 4°C for 3 days (control), the other half was stored at 4°C in a PAS/plasma mixture or in plasma alone (test) for 10 or 15 days. Subjects received radiolabeled platelet infusions on Day 3 (control) and Day 10 or 15 (test) to evaluate platelet recovery and survival. In addition to the in vivo platelet recovery and survival assays a number in vitro metabolic and functional platelet assays are performed on Day 3 and at the end of storage.
In April 2017 we revised our protocol. We changed our control comparator from a 3 day cold stored platelet unit to a fresh autologous platelet control comparator. Both the stored and the fresh platelets

were administered simultaneously using two different radioisotopes (≤15 μCi of indium for the stored and ≤20 μCi of chromium for the fresh). We used plasma, not PAS, as the storage solution. The study was re-titled Cold Apheresis Platelets in Plasma (CAPP). We soon discovered that the stored/indium signal was swamped by the fresh/chromium signal when calculations were performed in accordance with the 2005 Biomedical Excellence for Safer Transfusion (BEST) method. This approach yielded unusable data outputs when comparing products of very different signal strengths.

In May 2017 we modified the protocol to replace the chromium label with a second indium label of fresh platelets administered a week after the stored radiolabeled platelets. We are currently evaluating in vivo platelet recovery and survival assays of apheresis platelets stored for 5, 10 and 15 days at 4°C in comparison to fresh platelets. Additionally, we are comparing in vitro metabolic and functional platelet assays on the day of collection to those at the end of storage. As of 25-SEP-2017, only three of these datasets are complete so no conclusions can be drawn at this point. This study will continue with funding from another DoD award.

KEY RESEARCH ACCOMPLISHMENTS:

- Completion of study of platelets stored as whole blood at 4°C (Brrr Study)
- Publication in *Blood* – results of study of platelets stored as whole blood at 4°C
- Conclusion of study of apheresis platelets stored 4°C in different additive solutions and in plasma alone (CAPI Study)

CONCLUSION:

Brrr Study

Our study of platelets stored as whole blood at 4°C demonstrated that end-over-end rotation is required to reduce platelet adherence to the walls of the bag. Platelet yields in whole blood post-storage average 7.0 to 9.2 x 10^{10}. Thus, the FDA requirement of 5.5 x 10^{10} platelets/concentrate are easily met. At storage times between 10 to 15 days stored recoveries average 50% of fresh recoveries, stored survivals average >1 day, proposed post-storage criteria for whole 4°C stored platelets are met and based on in vitro measurements, the platelets are highly activated.

CAPI study

The poster presented to 2017 Military Health System Research Symposium, see appendix, gives the results of the radiolabeled autologous platelet recovery and survival data for our currently-completed studies.

In brief, the conclusions were:

- Storage in Intersol led to a significantly higher platelet yield after 10 day storage compared with plasma.
- Most in vitro platelet activation parameters did not differ significantly between 10d plasma, Intersol, and Isoplate. As expected, glucose and lactate were significantly lower in Intersol and Isoplate because of plasma removal.
- Post-storage recoveries for platelets stored in plasma or Intersol were significantly greater than for platelets stored in Isoplate or stored for 15 days in plasma.

- Platelets, stored for 10 days at 4ºC in plasma respond to agonists with inside out signaling and subsequent integrin activation, indicating that they could participate immediately in hemostatic processes.
- Platelet storage for 10 days at 4ºC in either plasma or Intersol could be used to expand the available supply of platelets to treat bleeding patients. We have nothing to report related to training and professional development or disseminating results to communities of interest.

A deeper understanding of the effects of cold storage on platelet function could greatly aid in improving the availability of platelets on the battlefield and in the civilian transfusion setting.

PUBLICATIONS, ABSTRACTS, and PRESENTATIONS:

- Slichter SJ, Fitzpatrick L, Jones MK, Pellham E, Bailey SL, Gettinger I. In vivo viability of platelets stored in whole blood at 4C. (Abstract) Blood 2015;126:2338.
- In Vivo Viability of Extended 10-Day 4ºC Stored Autologous Platelets - Report to 2017 Military Health System Research Symposium
- Manuscript for *Transfusion*, 'Viability and In Vitro Function of Platelets Stored in Whole Blood at 4° Centigrade', in development.

INVENTIONS, PATENTS AND LICENSES:
No inventions, patents, licenses or subcontracts were associated with this grant. No DD Form 882, "Report of Inventions and Subcontracts" is submitted.

REPORTABLE OUTCOMES: Nothing to report.

OTHER ACHIEVEMENTS: Nothing to report.

LISTING OF NONEXPENDABLE PERSONAL PROPERTY ACQUIRED WITH AWARD FUNDS:
No listing of nonexpendable personal property was acquired with award funds for this grant.

REFERENCES: None

APPENDICES:

- Federal Financial Report, Final SF425
- Final Technical Report
- Final Quad Chart
- Storage of Platelets in Whole Blood at 4°C Final Report
- In Vivo Viability of Extended 10-Day 4ºC Stored Autologous Platelets - Report to 2017 Military Health System Research Symposium

Quarterly Technical Progress Report Format
Front Cover

As of June 25, 2017 all funds related to this grant were exhausted. Therefore the Quarterly Technical Progress Report submitted July 7, 2017 is the final Quarterly Technical Progress Report.

Award Number:	W81XWH-13-2-0089
Log Number:	5779
Project Title:	Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON)
Principal Investigator Name:	Sherrill J. Slichter, MD
Principal Investigator Organization and Address:	Bloodworks Northwest (formerly Puget Sound Blood Center) 921 Terry Avenue, Seattle, WA, 98104
Principal Investigator Phone and Email:	206-689-6450, sherrills@BloodWorksNW.org
Report Date:	July 7, 2017
Report Period:	March 26, 2017 – June 25, 2017

Email the report and any other attachments to the Grants Officer's Representative (GOR) and Grants Specialist at the email addresses specified in the award document. Name the file with the award number, followed by "QtrlyTechProgReport Month Year."

If you have questions, contact the GOR.

1. Accomplishments: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.

1. Determine the optimum conditions for extended storage of autologous platelet concentrates in PAS.
    a. Identify an acceptable storage bag.
    b. Determine the best PAS-to-plasma ratio for platelet storage.
2. Evaluate the effects of Mirasol treatment of autologous WB on extended storage of PAS-stored platelet concentrates prepared from treated WB.
3. Determine the post-transfusion recovery and survival of pre-storage pooled extended stored platelet concentrates prepared from Mirasol-treated WB given to thrombocytopenic patients.

What was accomplished under these goals?
For <u>this quarterly reporting period only</u> describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided.

Due to instructions by Grants Officer's Representative work on this project was suspended. The Principal Investigator has been instructed that future studies must evaluate cold stored apheresis platelets.

Describe the Regulatory Protocol and Activity Status (if applicable).
Describe the Protocol and Activity Status for sections a-c, as applicable, using the format described for each section. If there is nothing significant to report during this reporting period, state "Nothing to Report."

(a) *Human Use Regulatory Protocols*

TOTAL PROTOCOLS: *State the total number of human use protocols required to complete this project (e.g., 5 human subject research protocols will be required to complete the Statement of Work."). If not applicable, write "No human subjects research will be performed to complete the Statement of Work."*

PROTOCOL(S): *List the identifier and title for all human use protocols needed to complete the project. Include information about the approved target number for clinical significance, type of submission, type of approval with associated dates, and performance status.*

The following format shall be used:

<u>Protocol (of total):</u>

Protocol [HRPO Assigned Number]:

Title:

Target required for clinical significance:

Target approved for clinical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IRB in addition to HRPO).

Status:

Report (i) progress on subject recruitment, screening, enrollment, completion, and numbers of each compared to original planned target(s), e.g., number of subjects enrolled versus total number proposed; (ii) amendments submitted to the IRB and USAMRMC HRPO for review; and (iii) any adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation.

TOTAL PROTOCOLS: 2

PROTOCOL (1 of 2 total):

Protocol : A-17951.2

Title: Cold Apheresis Platelets in Isoplate (CAPI).

Target required for clinical significance: 80 subjects may be enrolled to achieve 45 complete data sets

Target approved for clinical significance: 80

Note: This is an exploratory study. Clinical significance not implied.

SUBMITTED TO AND APPROVED BY:

- 1/11/16 – Full Approval from the Human Subjects Division of the University of Washington. Approval dates 12/16/15 – 12/15/16.
- 1/20/16 -Submission to Human Research Protection Office (HRPO) Office of Research Protections (ORP) United States Army Medical Research and Materiel Command.
- 3/2/16 .Screening questions from ORP-HRPO.
- 3/10/16 - Response from investigator (Dr. Slichter) to ORP HRPO
- 3/15/16 – Acknowledgement from ORP HRPO that answers were satisfactory.
- 4/4/16 - HRPO approval
- 6/13/16 – Problem Report submitted to Human Subjects Division of the University of Washington informing them that InterSol had been used, in error, instead of Isoplate for the first 5 subjects enrolled in the study.
- 7/11/16 - Minor non-compliance letter. Problem Report approved.
- 9/1/16 - Post Approval Verification and Education (PAVE) monitoring visit. (IRB sponsored)
- 9/7/16 - Protocol modification 3 approved by IRB. Pre-screening all subjects. 5 subjects each Isoplate, InterSol or none (plasma only). Acceptance criteria 40%, plasma only storage a standard double (not hyperconcentrated) will be collected.
- 9/7/16 – IND modification sent to FDA describing above changes to protocol.
- 10/26/16 - Modification 4 approved. May or may not agitate control or test platelets. Bloodworks Northwest intranet and internet recruitment wording.
- 11/30/16 - Modification 5 approved. Changes made in response to PAVE monitoring visit.
- 11/30/16 – Continuing Review Report (annual IRB review) approval. Approval dates 16 December 2016 – 15 December 2017.
- 12/15/16 - FDA IND 16680 Annual Report submitted.
- 12/16/16 – Annual report to HRPO submitted. Proposal Log Number/Study Number 13335012, Award Number W81XWH-13-2-0089, HRPO Log Number A-17951.2.

- 1/29/17 - Continuing Review Acceptance from Kimberly L. Odam, Human Subjects Protection Scientist, Human Research Protection Office.
- 4/6/17 - IRB approval of Mod 6. Submitted 3/13/17. Documents (Protocol – Consent) dated 3/1/17
- Submission included IRB Modification (see below), letter to FDA , Radiation Safety Application
    - Title changed to Cold Apheresis Platelets in Plasma (CAPP)
    - Discontinue use of the 3 day comparator
    - Fresh autologous platelet control comparator used as the control
    - Two different radioisotopes (≤15 µCi of indium and ≤20 µCi of chromium) The total radiation dose is approximately ≤40 µCi for a splenic dose of 6.1 mGy and a total body effective dose equivalent of 0.6 mSv.
    - Single unit (3.0 X 10^11/unit) instead of double
    - No PAS. Plasma will be the storage solution.
    - Platelet unit stored for 3 - 20 days
    - Fewer study visits and blood draws in total. Compensation reduced from $1000 to $700.
    - The total amount of whole blood collected is reduced by ~20 mL.
    - The apheresis collection volume is also decreased to about 350 mL.
    - 45 instead of 40 complete data sets
- 4/27/17 - Approved Modification 7 to add invitro testing on day of collection. Submitted 4/27/17. Protocol and consent version dates 4/20/17.
- **4**/28/17 - FDA approval to proceed with cold comparator modification. Phone message from Saundra Sonday. (Submission March 8, 2017).
- 5/25/17 - Modification #8 to IRB, Radiation Safety Office and FDA. Replace the Chromium label with a second Indium label administered a week after the first Indium-111 label. (The data was incalculable on the first two subjects when we injected chromium and indium labeled platelets simultaneously, because the signal strength of the fresh (chromium) labeled platelets overwhelmed the stored (indium) labeled platelets.) 17 instead of 12 study visits and more blood draws. Study participation will extend over a longer time period. Compensation to $900.
- 6/12/17 - Radiation Safety Office Approval for Mod #8.
- 6/28/17 - Final Approval for Modification #8. (note - three days beyond the reporting period of this quarterly report)

STATUS: As of June 25, 2017

(i) Number of subjects recruited/original planned target: 63/80
Number of subjects screened/original planned target: 63/80
Number of patients enrolled/original planned target: 39/80
Number of patients completed/original planned target: 25/80

(ii) Report amendments submitted to the IRB and USAMRMC HRPO for review:
Please see 'submitted to and approved by' above.

(iii) Adverse event/unanticipated problems involving risks to subjects or others and actions or plans for mitigation:
Nothing to report

PROTOCOL (2 of 2 total):

Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON) protocol withdrawn from consideration by HRPO and University of Washington IRB in October 2014. Study never approved or initiated.

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

"Cadaver" is defined as a deceased person or portion thereof, and is synonymous with the terms "human cadaver" and "post-mortem human subject" or "PMHS." The term includes organs, tissues, eyes, bones, arteries or other specimens obtained from an individual upon or after death. The term "cadaver" does not include portions of an individual person, such as organs, tissue or blood, that were removed while the individual was alive (for example, if a living person donated tissue for use in future research protocols, that tissue is not considered a "cadaver" under this policy, regardless of whether the donor is living or deceased at the time of tissue use).

***TOTAL ACTIVITIES**: State the total number of RDT&E, education or training activities that will involve cadavers. If not applicable, write "No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW)."*

***ACTIVITIES:** Provide the following information in a bulleted list for all RDT&E, education or training activities involving human cadavers conducted or supported during the quarter:*

- *Title of the RDT&E, education or training activity*
- *SOW task/aim associated with the activity*
- *Date the activity was conducted*
- *Identification of the organization's responsible individual (e.g., PI or individual primarily responsible for the activity's conduct)*
- *Brief description of the use(s) of cadavers in the activity and the total number of cadavers used during the reporting period*
- *Brief description of the Department of Army organization's involvement in the activity*
- *Status of document submission and approvals*
- *Problems encountered in the procurement, inventory, use, storage, transfer, transportation and disposition of cadavers used for RDT&E, education or training. Examples of problems include but are not limited to: loss of confidentiality of cadaveric donors, breach of security, significant deviation from the approved protocol, failure to comply with state laws and/or institutional policies and public relations issues.*

***TOTAL ACTIVITIES:** No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work (SOW).*

***ACTIVITES:** Not applicable.*

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S):

State the total number of animal use protocols required to complete this project (e.g., 2 animal use research protocols will be required to complete the Statement of Work.). If not applicable, write "No animal use research will be performed to complete the Statement of Work."

PROTOCOL(S):

List the identifier and title for all animal use protocols needed to complete the project. Include information about the approved target number for statistical significance, type of submission, type of approval with associated dates, and performance status.

The following format shall be used:

Protocol (_ of _ total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

Submitted to and Approved by:

Provide bullet point list of protocol development, submission, amendments, and approvals (include IACUC in addition to ACURO).

Status:

Provide bullet point list of performance and/or progress status relating to the above protocol and discuss any administrative, technical, or logistical issues that may impact performance or progress of the study (e.g. animal use protocol needs revision to minimize animal suffering, animal protocol modification to include additional staff) for the above ACURO approved protocol.

TOTAL PROTOCOL(S): *No animal use research will be performed to complete the Statement of Work."*

PROTOCOL (_ of _ total):

Protocol [ACURO Assigned Number]:

Title:

Target required for statistical significance:

Target approved for statistical significance:

SUBMITTED TO AND APPROVED BY:

STATUS:

2. What do you plan to do during the next reporting period to accomplish the goals and objectives?

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are actively enrolling subjects comparing apheresis platelet units stored in plasma for 10 days at 4°C to a fresh autologous platelet comparator collected and infused 1 week after the stored platelet infusion. We will continue our current recruiting and enrollment strategies, and analyze data as it becomes available.

3. Products: List any products resulting from the project during the reporting period. If there are no products to report for the current quarter, state "Nothing to report."

Examples of products include:

- *publications, conference papers, and presentations;*
- *website(s) or other Internet site(s);*

- *technologies or techniques;*
- *inventions, patent applications, and/or licenses; and*
- *other products, such as data or databases, biospecimen collections, germplasm, audio or video products, software, models, educational aids or curricula, instruments or equipment, data and research material, clinical or educational interventions, or new business creation.*

4. Participants & Other Collaborating Organizations

What individuals have worked on the project?
Provide the following information for: (1) Project Directors (PDs)/ PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.

Describe how this person contributed to the project. If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: *Mary Smith*
Project Role: *Graduate Student*
Researcher Identifier (e.g. ORCID ID): 1234567
Nearest person month worked: *5*
Contribution to Project: *Ms. Smith has performed work in the area of combined error-control and constrained coding.*

Name: *Sherrill J. Slichter, MD*
Project Role: *Principal Investigator*
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: *<0*
Contribution to Project: *Design, oversight and conduct of research study.*

5. Changes/Problems:

The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

a. Actual Problems or delays and actions to resolve them

Provide a description of current problems or issues that may impede performance or progress of this project along with proposed corrective action. Also describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

For an award that includes the recruitment of human subjects for clinical research or a clinical trial, discuss any problems or barriers encountered, if applicable, and what has been done to mitigate those issues. Discussion may highlight enrollment problems, retention problems, and actions taken to increase enrollment and/or improve retention.

Nothing to report

b. Anticipated Problems/Issues

Provide a description of anticipated problems or issues that have a potential to impede performance or progress. Also provide course of actions planned to mitigate problems or to take should the problem materialize.

Nothing to report

6. Special Reporting Requirements:

Quad Charts: If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.

Extended Storage of Pathogen-Reduced Platelet Concentrates (PRECON)

EDMS 5779/13335012

W81XWH-13-2-0089

PI: Sherrill J. Slichter MD **Org:** Bloodworks Northwest **Award Amount:** $866,326

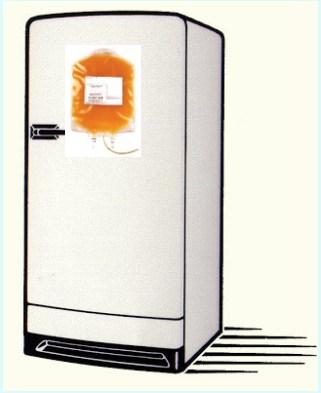
Study/Product Aim(s)

- Research related to cold storage of platelets derived from Whole Blood and Apheresis
- 4°C storage of platelets in Platelet Additive Solution (PAS),
- 4°C storage of extended storage of platelets in plasma

Approach

Study of apheresis platelets stored at 4°C in a plasma, entitled Cold Apheresis Platelets in Plasma (CAPP).

This is a non-clinical, exploratory study of recovery and survival of apheresis platelets stored in the cold (4°C).The comparator is a fresh autologous platelet aliquot. We are utilizing two sequential Indium-111 labels administered one week apart.



Refrigerated platelets in plasma- stored for up to 20 days

Accomplishment: Enrollment ongoing to evaluate apheresis platelets at 4°C

Timeline and Cost

Activities / CY	14	15	16	17
Study evaluating platelets in WB at 4°C				
Development and regulatory approval of apheresis platelets in PAS at 4°C study				
Apheresis platelets in PAS at 4°C study enrollment, data collection and analysis				
Apheresis platelets in plasma at 4°C study enrollment, data collection and analysis				
Estimated Budget ($K)	**$142**	**$220**	**$577**	**$866**

Updated: 07-JUL-2017

Goals/Milestones

CY16 Goal – Regulatory approval and study initiation (CAPI)

☑ Final IRB approval

☑ HRPO approval

☑ Enrollment, data collection and analysis

CY17 Goal – Continued enrollment, data collection and analysis

☑ Complete evaluation of Isoplate and InterSol cold stored platelets with and without agitation

☑ Enrollment, data collection and analysis (in progress)

☐ Compare apheresis platelets stored in plasma for 3-20 days at 4°C to same subject's fresh platelets.

Comments/Challenges/Issues/Concerns

CAPP is an exploratory study only. For confirmation of results the FDA requires a full set of in vivo platelet recovery/survival data and complimentary in vitro platelet quality data for 22-24 subjects for the selected cold storage period.

Budget Expenditure to Date

Projected Expenditure: $866,326

Actual Expenditure: $866,326

Final Report

Award Number W81XWH-12-1-0441, EDMS 5570

Log Number 11105004

"Storage of Platelets In Whole Blood at 4°C"

April 15, 2015

Introduction:

Since the 1970's, it has been known that platelet survivals are much better maintained at 22°C compared to 4°C while platelet recoveries are not significantly different based on storage temperature (Table 1).

Table 1

EFFECT OF STORAGE TEMPERATURE ON PLATELET VIABILITY

Number of Observations	STORAGE CONDITIONS		PLATELET	
	Time (Days)	Temperature (°C)	Recovery (%)	Survival (Days)
15	1	22	51 ± 3	8.2 ± 0.2
8	1	4	61 ± 7	1.3 ± 0.1
11	3	22	40 ± 3	7.9 ± 0.2
8	3	4	40 ± 5	1.0 ± 0.1

Legend: Platelet concentrates were prepared from normal subjects donated whole blood. After storage of the platelet concentrate, aliquots of the stored autologous platelets were labeled with ^{51}Cr, and the labeled platelets were injected into the platelet donor. Samples were obtained post-infusion to determine radiolabeled platelet recoveries and survivals. Data are reported as the average ±1 S.E.[(1)]

Approximately 80% of the platelets given in the U.S. are transfused into hematology/oncology patients where prolonged post-transfusion survivals of the stored platelets are important to decrease the need for frequent transfusions. Thus, the standard practice has been to store all platelets at 22°C regardless of the patient's clinical condition. However, for surgical/trauma patients, a short platelet life-span may be acceptable as these patients may only require immediate hemostasis until the vascular system can be repaired. The limited shelf-life of 5 days with 22°C storage severely limits platelet availability particularly at far-forward combat facilities, suggesting that we need to consider other options to support the platelet needs of these patients.

Increasingly, it has been recognized that trauma patients may be best supported with a ratio of 1 red cell, 1 plasma, and 1 platelet.[(2-3)] Therefore, the question becomes whether component therapy may be the best strategy to provide blood products for these patients or whether the transfusion of whole blood (WB) stored at 4°C would meet their needs. The major concern is the viability and function of platelets stored within WB at 4°C as platelets have never been stored in the cold for longer than 3 days and then only as platelet concentrates.

Purpose:

The purpose of this study was to determine the recovery and survival of autologous platelets that have been stored within WB for up to 22 days.

Primary Endpoint:

To determine how long autologous platelets can be stored in WB at 4°C with average post-storage platelet recoveries of ≥50% of the same donor's fresh platelet recoveries and platelet survivals of ≥1 day.

Experimental Design:

- Normal subjects donated a unit of WB.
- WB was stored at 4°C for 12 days (non-rotated) or for 10, 15, and 22 days (rotated end-over-end).
- At end of storage:
  - A platelet concentrate was prepared from the stored WB, and the platelets were labeled with ^{111}In.
  - A 50 ml blood sample was drawn from the subject, a fresh platelet sample was prepared from this blood, and the platelets were labeled with ^{51}Cr.
  - The subject was injected simultaneously with their autologous radiolabeled stored and fresh platelets.
  - Serial samples were drawn post-injection from the subject to determine the recovery and survival of the stored compared to the fresh platelets.

Results:

The first experiment was to store the WB obtained from 7 normal subjects for 12 days at 4°C without agitation until the end of storage. After storage, the WB was thoroughly mixed before a platelet concentrate was prepared (Table 2).

Table 2

12 DAY 4°C WHOLE BLOOD PLATELET STORAGE

Subject (#)	PLATELET RECOVERY (%)			PLATELET SURVIVAL (Days)		
	Fresh	Stored	% of Fresh	Fresh	Stored	% of Fresh
1	28	27	96*	8.1	2.4	30
2	50	15	30	4.9	1.9	38
4	63	19	30	9.8	1.9	19
5	73	27	37	8.7	1.3	15
6	41	35	85	8.5	2.5	30
7	53	15	28	8.9	1.5	16
8	44	14	32	8.9	1.1	12
Ave ±1 S.D.	50 ± 15	22 ± 8	48 ± 29	8.3 ± 1.6	1.8 ± 0.5	23 ± 10

*Without this result, average = 40 ± 22%.

There was a very wide standard deviation for the donors' platelet recoveries as a percentage of fresh. The first subject had an unexpectedly reduced fresh platelet recovery with a stored recovery that was 96% of the same donor's fresh recovery. Without the data from subject 1, platelet recoveries averaged only 40 ± 22% of the same donor's fresh recoveries. However, platelet survivals were all ≥1 day. In addition, 52 ± 12% of the donor's initial WB platelets were lost during storage (Table 3). These post-storage platelet results – both because of the poor platelet recoveries and platelet losses – were considered unacceptable.

Table 3

BASELINE SUBJECT DATA AND WHOLE BLOOD PLATELET AND HEMATOCRIT DATA

Subject (#)	Subject's HCT	Subject's Platelet Count (10^3/µl)	PLATELET COUNTS* Whole Blood ($x10^3$/µl) Pre-Storage	Post-Storage	Loss (%)**	TOTAL PLATELET COUNTS Whole Blood ($x\ 10^{10}$) Post-Storage	HEMATOCRITS Whole Blood Pre-Storage	Whole Blood Post-Storage
1	39	248	180	110	39	4.7	31	31
2	43	227	152	66	57	3.2	37	36
4	44	377	297	183	38	8.8	39	38
5	44	239	194	86	56	4.1	38	38
6	38	191	177	49	72	2.3	33	31
7	44	188	174	97	44	4.7	38	38
8	44	215	202	88	56	4.7	38	38
Ave ±1 S.D.	42 ± 3	244 ± 65	197 ± 47	97 ± 43	52 ± 12	4.6 ± 2.0	36 ± 3	36 ± 3

* Bag rotated end-over-end before platelet count obtained.
** Platelet loss during storage as a percent of the baseline platelet count.

Flow cytometry experiments demonstrated that the platelet loss during storage was not due to formation of either platelet aggregates or an excessive number of microparticles. Our hypothesis was that the platelets must be adhering to the walls of the bag to account for most of the platelet loss during storage. We then determined that, if the WB was rotated end-over-end during storage rather than only mixing the bag at the end of storage, 76 ± 4% of the initial platelets were maintained within the WB during 21 days of storage (Table 4).

Table 4

EVALUATION OF PLATELET LOSS DURING 4°C WB PLATELET STORAGE*

	STORAGE DAY (% of Baseline Platelet Count) 4	8	13	17	21	Ave ±1 S.D.
Rotation of WB End-Over-End During Storage	78	69	78	74	81	76 ± 4
Bench Top With Bag Rotated End-Over-End At End Of Storage	58	56	64	48	48	55 ± 7

*Two units of whole blood of the same blood type were pooled at baseline, mixed, and split into two bags for storage.

This constant end-over-end rotation of the WB during storage was then used for all future experiments. Platelet counts pre- and post-storage for up to 22 days confirmed the reproducibility of our original experiment (Table 5).

Table 5

WHOLE BLOOD ROTATED END-OVER-END DURING 4°C PLATELET STORAGE

Storage Time (Days)	N	WB PLATELET COUNTS x 10^{10} Baseline	WB PLATELET COUNTS x 10^{10} Post-Storage	Post-Storage Yield (% of Baseline)
10	10	9.5 ± 2.1	7.0 ± 1.4	74%
15	10	10.0 ± 1.0	7.6 ± 1.4	76%
22	3	12.9 ± 1.9	9.2 ± 0.7	71%

In vivo platelet recoveries and survivals were measured for platelets separated from rotated WB stored at 4°C for 10, 15, and 22 days (Table 6).

Table 6

WHOLE BLOOD ROTATED END-OVER-END DURING 4°C PLATELET STORAGE

Storage Time (Days)	N	PLATELET RECOVERY (%) Fresh	PLATELET RECOVERY (%) Stored	PLATELET RECOVERY (%) % of Fresh	PLATELET SURVIVAL (Days) Fresh	PLATELET SURVIVAL (Days) Stored	PLATELET SURVIVAL (Days) % of Fresh
10	10	50 ± 10	26 ± 7	51%	8.0 ± 1.0	1.3 ± 0.3	16%
15	10	55 ± 11	27 ± 11	49%	8.0 ± 0.1	1.2 ± 0.4	16%
22	3	62 ± 16	15 ± 1	25%	8.4 ± 0.6	1.1 ± 0.7	12%

Both platelet recoveries and survivals met our acceptance criteria for up to 15 days of storage but not for 22 days. Data on *in vitro* platelet assays are given in Table 7. These data demonstrate that cold stored platelets are highly activated, suggesting that these platelets may provide immediate hemostasis for actively bleeding patients.

Table 7

***IN VITRO* ASSAYS OF ROTATED WHOLE BLOOD 4°C PLATELET STORAGE**

Storage Time (Days)	N	Microparticles (%) Pre	Microparticles (%) Post	Annexin V Binding (% Positive) Pre	Annexin V Binding (% Positive) Post	P-Selectin (% Positive) Pre	P-Selectin (% Positive) Post	TGT Peak (nM Thrombin) Pre	TGT Peak (nM Thrombin) Post
10	10	1.9 ± 0.9	21.7 ± 19.0	6 ± 6	32 ± 13	11 ± 11	68 ± 17	63 ± 25	177 ± 74
15	10	1.2 ± 0.9	20.0 ± 10.4	5 ± 6	51 ± 21	10 ± 11	76 ± 10	83 ± 16	162 ± 37
22	3	1.0 ± 0.8	36.3 ± 6.3	10 ± 8	56 ± 7	7 ± 2	92 ± 1	109 ± 62	171 ± 54

Conclusions:

- End-over-end rotation of WB during 4°C storage is required to reduce platelet adherence to the walls of the bag.
- Platelet yields in the WB post-storage average 7.0 to 9.2 x 10^{10}. Thus, the FDA requirement of 5.5 x 10^{10} platelets/concentrate are easily met.

- At storage times between 10 to 15 days:
  - Stored recoveries average 50% of fresh recoveries.
  - Stored survivals average >1 day.
  - Proposed post-storage criteria for WB 4°C stored platelets are met.
- Based on *in vitro* measurements, the platelets are highly activated.

Future Studies:

It will be necessary to document the hemostatic efficacy of platelets stored within WB at 4°C. This will likely require large transfusion trials monitoring bleeding outcomes in surgical or trauma patients.

References:

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2. Borgman MA, Spinella PC, Perkins JG, *et al*. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma 2007;63:805-813.
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In Vivo Viability of Extended 10-Day 4ºC Stored Autologous Platelets

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Abstract:

Background: The limited 5-day storage time of room temperature (22˚ C) stored platelets severely limits platelet availability at far-forward combat medical facilities and rural civilian hospitals. Prior studies comparing 3-day 22ºC versus 4ºC stored platelets showed comparable post-transfusion platelet recoveries of 40 ± 3% and 40 ± 5%, respectively, but survivals were significantly different 7.9 ± 0.2 days and 1.0 ± 0.1 days (p<0.01). However, for the treatment of actively bleeding patients requiring immediate hemostasis, platelet survivals of 1 day or less may be adequate. Methods: Nineteen normal subjects had a 2-unit apheresis procedure. One unit was stored at 4ºC in plasma for the FDA approved 3 days ("control unit"). The other "test" unit was stored for 10 days in plasma (n=6) or it was hyperconcentrated during collection and re-suspended in 35% plasma with either Intersol (n=5) or Isoplate (n=3) at 65%. An additional group was stored for 15 days in plasma (n=5). At the beginning and end of non-agitated storage, samples were drawn for in vitro parameters. At the end of non-agitated storage both units were radiolabeled with ^{111}Indium and infused into their respective donors. Pre- and post-infusion samples were drawn to determine platelet recoveries and survivals. Results: Platelet counts of the "control" units averaged 3.59 ± 0.31 x 10^{11} pre storage, and 3.57 ± 0.38 x 10^{11} post-storage (99 ± 3% of pre-storage). For the "test" plasma, Intersol, and Isoplate, and 15 day plasma units, post-storage platelet counts averaged 2.86 ± 0.39 x 10^{11}, 2.82 ± 0.35 x 10^{11}, 2.52 ± 1.28 x 10^{11}, and 2.42 ±0.36 x 10^{11} , respectively (80 ± 7%, 107 ± 12%, 90 ± 39%, and 72 ±14 % of pre-storage values). Most in vitro parameters did not differ significantly between 10 day stored plasma, Intersol, and Isoplate, except for glucose and lactate, which may be due to plasma removal. 15 day plasma storage led to significantly more microparticle formation. For the "control" units, post-storage recoveries averaged 43 ± 11% and survivals 2 ± 0.4 days. For the "test" 10 day plasma, Intersol, Isoplate, and 15 day plasma units, post-storage recoveries averaged 24 ± 8%, 18 ± 4%, and 8 ± 2%, and 11 ± 3% respectively (55 ± 11%, 43 ± 6%, 21 ± 8%, and 30 ± 3% of the same subject's 3-day data). As a percentage of their 3-day recoveries, both the plasma and Intersol units were significantly greater than the Isoplate units (p<0.001 and p<0.05, respectively), but there were no differences between the plasma and Intersol units. Post-transfusion survivals for the 10-day platelets stored in plasma, Intersol, Isoplate, and 15 day stored in plasma averaged 1.2 ± 0.3 days, 1.1 ± 0.3 days, 0.9 ± 0.8 days, and 0.7 ± 0.2 days respectively (59 ± 12%, 56 ± 8%, 48 ± 42%, and 36 ±7% of the same subject's 3-day data). There were no significant differences in platelet survivals. Platelets stored for 10 days showed the ability to respond to agonists with integrin activation, which required active inside out signaling and integrin conformational change, indicating that they could contribute to hemostasis in vivo immediately upon transfusion.

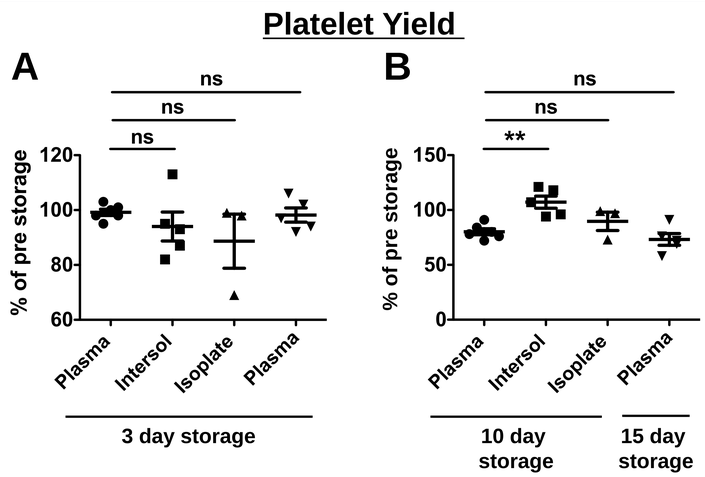
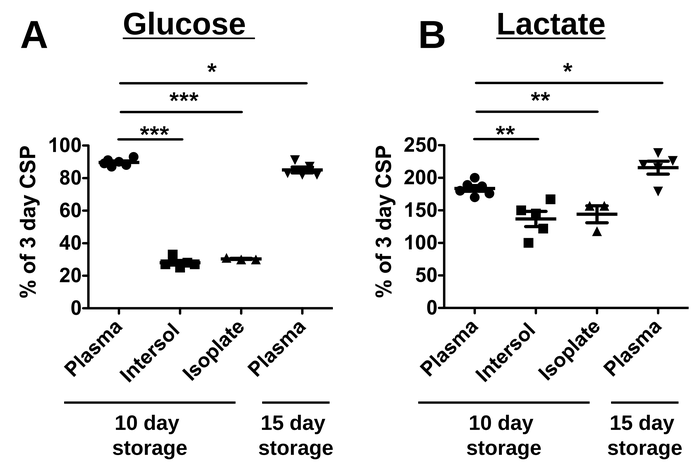


Figure 1: Actual platelet yield [Total platelet yield in the component, calculated by multiplying the platelet count of the sample times the volume of the component (platelet count x component volume = actual platelet yield)]. Platelets were stored in either plasma alone (Plasma, black circles and downward triangles), or in a 65% platelet additive solution, 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles). **A**, Platelets were stored for three days at 4ºC. Results are shown as percentage of pre-storage (fresh). No significant differences were seen. **B**, 10 day and 15 day stored (as indicated) platelets as percentage of 3 day platelets. ns= not significant, **p=0.0012.



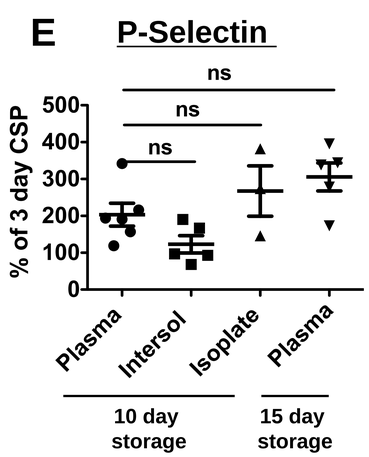


Figure 2: In vitro parameters: Platelets were stored in either plasma alone (Plasma, black circles and downward triangles), or in a 65% platelet additive solution, 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles) at 4ºC, for either 10 or 15 days as indicated. **A**, glucose and **B**, lactate determined by blood gas analysis. **C**, CD61-positive microparticles determined by flow cytometry. **D**, Annexin V positive events measured by flow cytometry. **E**, CD62P-positive (P-selectin) events measured by flow cytometry. All results are shown as percentage of 3 day 4ºC-stored platelets. *p<0.05, **p<0.01, ***p<0.0001, ns= not significant.

The authors have no conflict of interest to disclose

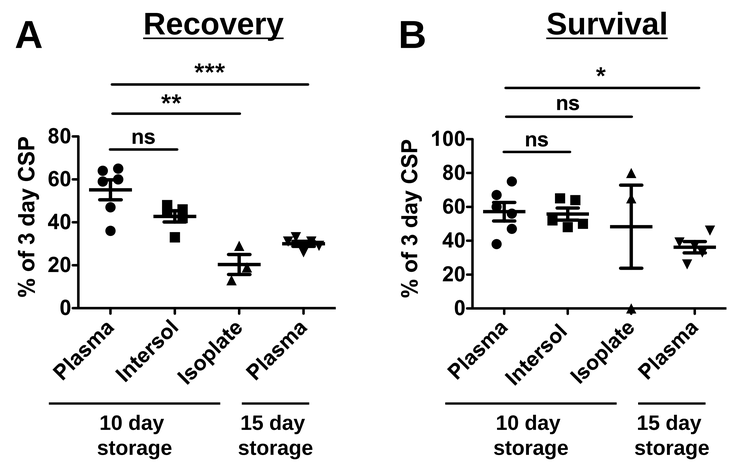


Figure 3: In vivo parameters: Healthy human subjects received autologous platelet transfusions. stored at 4ºC. (Plasma, black circles and downward triangles), or in a 65% platelet additive solution, 35% plasma mixture (Intersol, black squares, or Isoplate, black, upward triangles). **A**, Recovery of transfused platelets at 1 hour time point. **B**, Survival of transfused platelets. All results given as percentage of 3 day 4ºC-stored platelets. *p<0.05, **p<0.01, ***p<0.0001, ns= not significant.

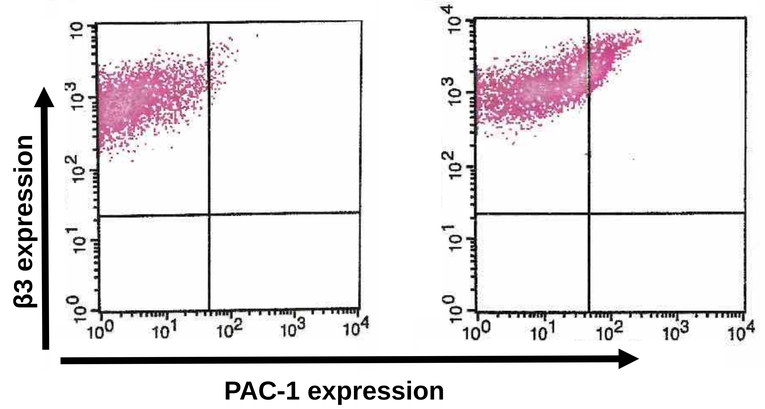


Figure 4: Platelet-integrin activation: 4ºC-stored platelets were stored for 10 days and either left unstimulated (**A**, baseline, left panel) or stimulated with 10uM ADP (final concentration) (**B**, ADP, right panel). The activation-dependent αIIbβ3-integrin antibody PAC-1 was incubated with both samples along with a activation independent β3-chain antibody (Y-axis, stains all platelets).

Conclusions:

- **Storage in Intersol led to a significantly higher platelet yield after 10 day storage compared with plasma.**
- **Most in vitro platelet activation parameters did not differ significantly between 10d plasma, Intersol, and Isoplate. As expected, glucose and lactate were significantly lower in Intersol and Isoplate because of plasma removal.**
- **Post-storage recoveries for platelets stored in plasma or Intersol were significantly greater than for platelets stored in Isoplate or stored for 15 days in plasma.**
- **Platelets, stored for 10 days at 4ºC in plasma respond to agonists with inside out signaling and subsequent integrin activation, indicating that they could participate immediately in hemostatic processes.**
- **Platelet storage for 10 days at 4ºC in either plasma or Intersol could be used to expand the available supply of platelets to treat bleeding patients.**